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(54) Title: RIGID LIPOSOMAL COCHLEATE AND METHODS OF USE AND MANUFACTURE

(57) Abstract: Employing liposomes having a high transition temperature at least partially disposed in a matrix, compositions are provided that can be used to deliver one or more cargo moieties, e.g., a drug, a nutrient, an imaging agent and/or non-steroidal anti-inflammatory. The matrix can be a lipid precipitate and/or a cationic bridge. Methods of making and using these compositions preferably cochleates, are also disclosed.

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RIGID LIPOSOMAL COCHLEATE AND METHODS OF USE AND MANUFACTURE

Related Applications

This application claims the benefit of U.S. Provisional Application Nos. 60/531,546, filed December 19, 2003 and 60/565,120, filed April 23, 2004, which applications are incorporated herein by this reference.

Technical Field

The invention generally relates to compositions including high transition temperature liposomes at least partially disposed in a matrix.

Background

Conventional liposomal suspensions have fluid membranes that can allow encapsulated drugs to leak out over time. Moreover, upon dehydration or exposure to a cationic environment, the lipid bilayers of a liposome can collapse, displacing the aqueous interior of the liposomes, including the encapsulated drug, and exposing the drug to the environment. Consequently, conventional liposomes may inefficiently retain encapsulated drug, exposing the drugs to environmental conditions that can lead to degradation or deactivation.

Summary of the Invention

The present invention provides stable liposome compositions for the delivery of a variety of cargo moieties (*e.g.*, drugs and/or nutrients). The liposomes are formulated such that they are rigid at ambient temperatures. The rigid liposomes preferably are at least partially disposed in a matrix, such as a cation bridge or lipid precipitate, that can serve to further stabilize the composition and/or carry additional cargo moieties.

One advantage of such liposome compositions is that cargo moieties associated with the rigid liposomes of the invention (*e.g.*, residing within the liposomal aqueous interior and/or associated with the lipid bilayer) cannot readily

dissociate from the liposome. The invention is particularly advantageous when a cargo moiety is disposed in the aqueous interior of the liposomes, which typically would be displaced upon dehydration and/or addition of cation. When the rigid liposomes of the invention are exposed to conditions in which conventional liposomes would collapse and/or precipitate, the rigid liposomes do not collapse and the associated cargo moieties are retained and protected.

Another advantage of the compositions of the present invention is that they can be formulated so that the transition temperature is at or below the body temperature of an animal such that the rigid liposomes will become fluid after ingestion, facilitating delivery of cargo moieties to the animal. Another advantage of the current invention is that a matrix can be used to deliver cargo moieties as well. The cargo moieties can exist, *e.g.*, in a cationic bridge or a lipid bilayer of a precipitate.

Brief Description of the Drawings

Figure 1A is an image of crystalline distearoyl phosphatidylserine (DSPS) upon direct addition to water and Rhodamine labeled Dextran (Rho-Dextran) at a temperature below its transition temperature (68°C).

Figure 1B is an image of fluid DSPS liposomes formed upon heating and periodic vortexing of the crystalline DSPS depicted in Figure 1A above the transition temperature.

Figure 1C is an image of an exemplary composition of the invention including the rigid DSPS liposomes of Figure 1B, at a temperature below its transition temperature and disposed in a calcium matrix.

Figure 1D is an image of the composition of Figure 1C after the addition of EDTA at a temperature below the lipid bilayer transition temperature. The EDTA did not appear to induce an observable change in the structure.

Figure 2A is an image of rigid DSPS Rho-Dextran liposomes disposed in a matrix formed from DOPS liposomes precipitated with calcium. The image was captured with phase contrast microscopy. The image indicates that the Rho-Dextran is maintained within the rigid liposomes.

Figure 2B is an image of the composition of Figure 2A captured by employing both fluorescence and phase contrast microscopy.

Figure 2C is an image of the composition of Figure 2A captured by employing fluorescence microscopy.

Detailed Description of the Invention

In conventional liposomes, fluidity of the lipid bilayer can contribute to leaking or loss of cargo moieties from their interior and/or the bilayer. Membrane fluidity, however, is a key factor in the successful fusion of liposomes and lipid precipitates (*e.g.*, cochleates) with membranes. A new composition has been discovered that provides stability at ambient temperatures and fluidity at high transition temperatures (*e.g.*, the body temperatures of animals such as humans). Such a composition provides a delivery vehicle stable at temperatures normally experienced during storage and shipping but that is capable of delivery upon administration to a subject.

The lipid structures of the present invention are particularly advantageous because they are capable of retaining cargo moieties which can leak out of conventional liposomes and potentially, in some instances, cochleates, *e.g.*, hydrophilic cargo moieties. Such cargo moieties are retained in the lipid structure until it is heated to above the lipid bilayer transition temperature because of the rigidity of the system at temperatures lower than the transition temperature.

In one aspect, the composition includes a plurality of liposomes having a high transition temperature, *i.e.*, "rigid liposomes," at least partially disposed in a matrix. In one embodiment, the matrix is a cation bridge between at least a portion of the plurality of rigid liposomes. Surprisingly, in these embodiments, even addition of a chelating agent, which typically would open up a lipid precipitate, does not induce a change in the structure. When heated above the transition temperature in the presence of chelating agent, however, the structure opens up and the liposomes are capable of fusion.

Additionally or alternatively, the rigid liposomes can be disposed in a matrix including lipid precipitate including a charged lipid and a multivalent counter ion, *e.g.*, a cochleate formed from multivalent cation and liposomes including negatively charged lipid. The lipid precipitate also can include additional cargo moieties and/or can have, but is not limited to a high transition temperature.

In order to more clearly and concisely describe the subject matter of the claims, the following definitions are intended to provide guidance as to the meaning of specific terms used in the following written description, examples and appended claims.

A "liposome" is a structure composed of at least one lipid bilayer disposed about a typically aqueous volume. Liposomes can be formed from a wide variety of components well known in the art. For example, liposomes may include lipids such as phospholipids, and also may include other lipids such as cholesterol.

The term "transition temperature" refers to the temperature at which a lipid bilayer begins to transition from a rigid to a fluid state. The term "high transition temperature" refers to a lipid bilayer transition temperature that is higher than at least about 20°C. Liposomes with lipid bilayers having a high transition temperature are referred to herein as "rigid liposomes" as they are less fluid at ambient temperatures than conventional liposomes that have lower transition temperature lipid bilayers.

The term "precipitate," as used herein, refers to precipitates of charged lipid and multivalent counter ion. A precipitate can be a cochleate if it takes on an alternating cationic and lipid bilayer structure. However, a precipitate can have alternative structures as well.

As used herein, the term "cochleate" refers to lipid precipitates that include alternating lipid bilayer and counter-ion sheets, stacked and/or rolled up with little or no internal aqueous space. The term "encochleated" means associated with a cochleate structure, *e.g.* by incorporation into the cationic sheet, and/or inclusion in the lipid bilayer. Methods of making and using cochleates are described, *e.g.*, in U.S. Patent Nos. 5,994,318 and 6,153,217, which are incorporated in their entirety by this reference. Accordingly, an encochleated rigid liposome would include at least one rigid liposome disposed at least partially in a cochleate matrix.

As used herein, the term "multivalent cation" refers to a divalent cation or higher valency cation, or any compound that has at least two positive charges, including mineral cations such as calcium, barium, zinc, iron and magnesium and other elements capable of forming ions or other structures having multiple positive charges capable of chelating and bridging negatively charged lipids. Additionally or alternatively, the multivalent cation can include other multivalent cationic

compounds, *e.g.*, cationic cargo moieties. The term "divalent metal cation," as used herein, refers to a metal having two positive charges.

The term "matrix" refers to a component that binds at least two liposomes. The matrix can be, *e.g.*, an ionic bridge between two liposomes that contain charged lipid, *e.g.*, a multivalent cationic bridge between negatively charged lipid in the high transition temperature liposomes. Additionally or alternatively, the matrix can be a lipid-cation precipitate, for example a cochleate component and/or a precipitate of high transition temperature liposomes and a multivalent cation, optionally incorporating liposomes that do not have a high transition temperature.

A "cargo moiety" is a moiety associated with the compositions of the invention, and generally does not refer to the lipid employed to form the liposomes or precipitates. Cargo moieties also include any compounds having a property of biological interest, *e.g.*, ones that have a role in the life processes of a living organism. A cargo moiety may be organic or inorganic, a monomer or a polymer, endogenous to a host organism or not, naturally occurring or synthesized in vitro and the like. In some embodiments, the cargo moiety does not refer to the counterion employed to form the matrix.

In one aspect, the present invention provides a composition that generally includes a plurality of liposomes having a high lipid bilayer transition temperature at least partially disposed in a matrix. These "rigid liposomes" are less fluid at ambient temperatures than conventional liposomes that have lower lipid bilayer transition temperatures.

The fluidity of a lipid bilayer depends on both its composition and temperature, as is readily demonstrated in studies of synthetic bilayers. A synthetic bilayer made from a single type of phospholipid transitions from a rigid crystalline state to a liquid state at a characteristic transition point or lipid bilayer transition temperature. When there is more than one type of lipid and/or additional components present in the lipid bilayer, the lipid bilayer transition can occur over a temperature range, gradually becoming less fluid as the temperature decreases. The lipid bilayer transition temperature is the temperature at which the transition from solid to liquid begins.

A practitioner can readily determine the transition temperature of lipids and lipid mixtures from, *e.g.*, product literature, lipid databases, and routine experimentation. For example, lipids having a high transition temperature can be identified readily employing on-line databases, such as the lipid databases maintained by Ohio State University (http://www.lipidat.chemistry.ohio-state.edu) and Avanti Polar Lipids (www.avantilipids.com).

Additionally, the transition temperature of a lipid bilayer readily can be determined by heating a lipid or lipid mixture from below the transition temperature while observing the transition under a microscope. The lipid bilayer transition temperature can be readily adjusted by employing other lipids or cholesterol. While transition temperatures of a large number of lipids are readily available, a general knowledge of the affect of structure on transition temperatures can also be employed to choose lipids. Generally, the shorter the hydrocarbon chain/or and the more double bonds, the lower the transition temperature. A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another, and cis-double bonds produce kinks in the hydrocarbon chains that make them more difficult to pack together, so that the membrane remains fluid at lower temperatures. Moreover, the head group of a fatty acid can affect the transition temperature (*e.g.*, a choline head group lowers the transition temperature relative to an ethanolamine head group). Additional substituents, *e.g.*, cholesterol, can broaden the range of transition because they inhibit regularity in the bilayer. Employing the teachings contained herein, the skilled practitioner can readily manufacture the compositions of the invention using no more than routine experimentation.

Accordingly, in some embodiments, the liposomes include one or more saturated phospholipids and/or phospholipids having a chain length of 16 to 24 carbons.

In one embodiment, the transition temperature of the lipid bilayer is at least about 30°C. In one embodiment, the transition temperature is at least about 45°C and in another embodiment, the transition temperature is at least about 60°C. In certain embodiments, the transition temperature is below about 100°C In some embodiments, the lipid bilayer transition temperature is between about 25°C and 95°C. In other embodiments, the lipid bilayer transition temperature is between

about 30°C and 85°C. In yet other embodiments, the lipid bilayer transition temperature is between about 35°C and 75°C. In one embodiment, the transition temperature of the lipid bilayer is lower than body temperature, *e.g.*, below about 40°C.

In other embodiments, the transition temperature is at least about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 ... 100°C. All individual values and ranges within this range are considered to be embodiments of the invention and can be chosen based on the specific manufacturing, storage, and/or delivery conditions to which the compositions will be exposed. For example, the transition temperature can be chosen to be higher than storage conditions but lower than or about equal to the body temperature of an animal to which the compositions will be administered.

In another embodiment, the transition temperature of the lipid bilayer is higher than a human or other animal body temperature. One advantage of such a rigid lipid structure is that the structure may not allow fusion such that, *e.g.*, imaging agents are not released but retained within the rigid lipid structure.

In one embodiment, the liposomes include a majority of charged lipid. In certain embodiments, the liposomes of the invention include at least about 75%, at least about 80%, at least about 90%, or at least about 95%, charged lipid. In some embodiments, the liposomes include one or more phospholipids. In one embodiment, the phospholipid is a charged phospholipid. The liposomes can include negatively charged, positively charged and/or neutral phospholipids. In certain embodiments, the lipid is a negatively charged phospholipid.

In one embodiment, the liposomes include at least one phospholipid selected from the group consisting of: distearoyl phosphatidylserine (DSPS), distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidic acid (DPPA), dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidylserine (DPPS), dipalmitoyl phosphatidylgycerol (DPPG), dimyristoyl phosphatidylethanolamine (DMPE), dioleoyl phosphatidic acid (DOPA), and dimyristoyl phosphatidylserine (DMPS).

In certain embodiments, the rigid liposomes are disposed, at least in part, in a matrix. In one embodiment, the matrix includes an ionic bridge between the rigid liposomes. The ionic bridge can be a multivalent ion that having a charge

opposite that of some of the lipids employed to form the rigid liposomes. For example, where the plurality of liposomes includes a negatively charged lipid, the matrix can be a cationic bridge between a multivalent cation and the negatively charged lipid. Similarly, where the plurality of liposomes includes a positively charge lipid, the matrix can be an anionic bridge between a multivalent anion and the positively charged lipid.

In some embodiments, more than one, *e.g.*, two or three or more, populations or formulations of rigid liposomes may be disposed in a matrix. For example, a matrix may incorporate rigid liposomes which include a first lipid and a first cargo moiety and rigid liposomes which include a second lipid and a second cargo moiety. Alternatively, a matrix may incorporate rigid liposomes which include a first lipid and a first cargo moiety and rigid liposomes which include the first lipid and a second cargo moiety. In yet another example, a matrix may incorporate rigid liposomes which include a first lipid and a first cargo moiety and rigid liposomes which include a second lipid and the first cargo moiety. Such formulations may be advantageous, *e.g.*, for coadministration of two or more cargo moieties or for varying release profiles.

Additionally or alternatively, the matrix can include a precipitate that generally includes a multivalent ion and a charged lipid (*e.g.*, a multivalent cation and a negatively charged lipid). The matrix can be a cochleate or other structure. The lipid can include, but is not limited to, high transition temperature lipids. The precipitate can include an ionic bridge between the precipitate and the rigid liposomes. Alternatively, the matrix can be disposed about the liposome without a chemical, ionic or other bridge or bond.

In another aspect, the present invention includes methods for forming the compositions of the present invention. The method generally includes forming a matrix at least partially about a plurality of liposomes having a high transition temperature. In one embodiment, the plurality of liposomes includes an anionic lipid and the matrix comprises a multivalent cation. The matrix can include a cationic bridge formed by the multivalent cation between the liposomes. Additionally or alternatively, the matrix can include a precipitate of the multivalent cation and a second plurality of liposomes. In a preferred

embodiment, the precipitate is a cochleate that includes the multivalent cation and a second plurality of liposomes

In one embodiment, a matrix can be formed by introducing at least one multivalent ion (*e.g.*, calcium) to the rigid liposomes. In specific embodiments, the multivalent ion is cationic and the liposomes include negatively charged lipids. A unique and surprising structure has been observed to form upon addition of multivalent cation to rigid liposomes including negatively charged lipid. The multivalent ion forms an ionic bridge between the rigid liposomes. In the examples and figures disclosed herein, the structure appears under a microscope as a grape-like cluster of liposomes encrusted or otherwise held together by the cation. Also surprising about this structure, is that the addition of a chelating agent (*e.g.*, EDTA) does not open up the matrix. Without wishing to be bound by any particular theory, it is believed that the bridge between the multivalent ion and the high-transition temperature lipids in the liposomes is "locked" because the lipid does not allow the chelating agent to access the multivalent ion.

In another embodiment, the matrix is formed by introducing or forming a plurality of liposomes that have a lower lipid bilayer transition temperature and precipitating these liposomes with a counter ion. Such liposomes and resulting precipitates can include lipids, lipid mixtures, cargo moieties, cations and other components described, *e.g.*, in WO 2004091579, published October 28, 2004 and entitled "Novel Encochleation Methods, Cochleates, and Methods of Use" and in WO 2004091579, published October 28, 2004 and entitled "Cochleate Compositions Directed Against the Expression of Proteins," both of which are incorporated herein by this reference in their entireties. The methods described in WO 2004091579 and WO 2004091579 can also be readily employed in connection with the present invention. The precipitate optionally can be formed in the presence of additional cargo moieties such that the cargo moieties are incorporated into the matrix. These cargo moieties can be the same or different from the cargo moieties associated with the rigid liposomes. The ion can also form an ionic bridge between the precipitate and the liposomes.

For example, one or more multivalent cations can be introduced to suspension including a plurality of rigid liposomes and a plurality of liposomes having a lower lipid bilayer transition temperature, both including negatively

charged lipid. In certain embodiments, the multivalent cation precipitates with calcium about the liposomes. In other embodiments, the multivalent cation also forms a bridge with the negatively charged lipid in the rigid liposomes.

In still other embodiments, a matrix may be formed by introducing a plurality of liposomes which include a negatively charged lipid to a plurality of liposomes that have a high transition temperature include little or no negatively charged lipid, and precipitating the negatively charged lipid with a counter ion. Without wishing to be bound by any particular theory, it is believed that the rigid liposomes would not interact with the counter ion, but may be at least partially trapped within the matrix.

In another embodiment, the matrix includes matrix portions that are precipitates and/or cochleates, and portions that are cationic bridges between rigid liposomes.

In one embodiment, the matrix is disposed completely or substantially about the plurality of liposomes having a high transition temperature. In another embodiment, the matrix is only partially disposed about the liposomes. In some embodiments, the matrix is formed in several steps, *e.g.*, forming a first precipitate with a cargo moiety, and then forming a second precipitate with another cargo moiety. Multiple variations of this method can be employed and are well within the scope of this invention. For example, the rigid liposomes including a first cargo moiety can be at least partially disposed in a matrix that is a cationic bridge and includes a second cargo moiety, subsequently a further precipitate can be formed with a plurality of liposomes, cation and a third moiety. Another example is the use of at least two different types of liposomes for forming the precipitate with different lipid content and/or different cargo moieties associated with each.

In one embodiment the method includes the step of forming of a plurality of liposomes with a high lipid bilayer transition temperature. The liposomes can be formed, *e.g.*, by heating an aqueous mixture of lipids above the lipid bilayer transition temperature. Alternatively, the lipid can be added to an aqueous media at a temperature above the lipid bilayer transition temperature. In certain embodiments, the mixture is mixed, sonicated, or vortexed to hasten the formation of liposomes.

In one embodiment, the method includes introducing at least one cargo moiety for association with the rigid liposomes. Cargo moieties can be associated with the structures in a variety of ways. For example, the cargo moieties can be disposed in the interior of the rigid liposomes and/or associated with the rigid liposome bilayer. Generally, hydrophilic moieties will be entrapped in the liposomal aqueous interior, and moieties having both hydrophilic and hydrophobic portions will associate with the lipid bilayer. Hydrophobic moieties can associate with the lipid bilayer and/or can exist in emulsion in the aqueous interior of the liposomes.

The rigid liposomes can include rigid liposomes with different lipid mixtures and/or different cargo moieties. This can be achieved by a variety of methods, *e.g.*, by mixing the different types of rigid liposomes together after formation and/or solidification. This also can be achieved, *e.g.*, by forming a first plurality of liposomes having a first lipid bilayer transition temperature, cooling below the first transition temperature, forming a second plurality of rigid liposomes having a second lipid bilayer transition temperature below the first transition temperature, and cooling below the second transition temperature. The same or different cargo moieties can be introduced for association with each plurality of liposomes.

In one embodiment, liposomes with a high lipid bilayer transition temperature are formed primarily or exclusively from DSPS, which has a transition temperature of about 68°C. When heated above its transition temperature in aqueous solution including a desired cargo moiety, liposomes form than include an aqueous solution including cargo moiety. When the liposomes are cooled below the lipid bilayer transition temperature, *e.g.*, room temperature, the liposomes become rigid.

The liposomes can optionally then be washed or otherwise processed to remove all or substantially all of the cargo moiety that is not associated with the rigid liposomes. Such a step can be desirable, *e.g.*, when the cargo moiety is toxic or inflammatory and/or so that it can be recycled into another batch of rigid liposomes. Additionally or alternatively, the liposomes can be washed after the formation of the matrix. These compositions can be particularly advantageous, *e.g.*, in oral preparations where the cargo moiety is toxic or otherwise harmful to

the GI tract and/or the cargo moiety is susceptible to degradation in the GI tract, because the cargo moiety will not be released from the composition until after it passes into the bloodstream. Accordingly, the GI tract is protected from the cargo moiety and the cargo moiety is protected from the GI tract.

Additionally or alternatively, cargo moieties can be associated with the matrix. For example they can be disposed in a cationic bridge (*e.g.*, a calcium cation bridge formed between rigid liposomes), or in a lipid-cation precipitate (*e.g.*, in a cochleate matrix disposed at least partially about a plurality of rigid liposomes). The structure can have more than one cargo moiety incorporated in more than one manner. For example, one type of cargo moiety can be disposed with a cochleate matrix for more immediate delivery, and a second type of cargo moiety can be disposed in the rigid liposome structure for later delivery.

Additionally or alternatively, cargo moieties can be disposed in conventional liposomes included (*e.g.*, in an emulsion including rigid liposomes) in the compositions of the present invention. While typically, such delivery is less efficient, it may be useful in some circumstances (*e.g.*, when loss of cargo moiety in the conventional liposomes is desired and/or acceptable). By way of example, a composition of the invention might include a drug that is harmful to the stomach that is entrapped within rigid liposomes and a second drug or other agent that protects the stomach disposed in conventional liposomes (*i.e.*, liposomes that do not have a high transition temperature) and/or a cochleate matrix. Numerous other variations readily can be envisioned by a practitioner based on the present specification.

Classes of cargo moieties include but are not limited to vitamins, minerals, nutrients, micronutrients, amino acids, toxins, microbicides, microbistats, co-factors, enzymes, polypeptides, polypeptide aggregates, polynucleotides, lipids, carbohydrates, nucleotides, starches, pigments, fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, flavorings, essential oils, extracts, hormones, cytokines, viruses, organelles, steroids and other multi-ring structures, saccharides, metals, metabolic poisons, antigens, imaging agents, porphyrins, tetrapyrrolic pigments, drugs and the like.

The cargo moiety can be a diagnostic agent, such as an imaging agent. Imaging agents include nuclear agents and porphyrins. Porphyrins include

tetrapyrrolic agents or pigments. One such tetrapyrrolic agent is Zinc Tetra-Phenyl Porphyrin (ZnTPP), which is a hydrophobic, fluorescent molecule that has high absorption in the visible spectrum (dark purple).

The cargo moiety can be a polynucleotide that is expressed to yield a biologically active polypeptide or polynucleotide. Thus, the polypeptide may serve as an immunogen or, *e.g.*, have enzymatic activity. The polynucleotide may have catalytic activity, *e.g.*, be a ribosome, or may serve as an inhibitor of transcription or translation, *e.g.*, a small interfering RNA (siRNA) or an antisense molecule. The polynucleotide can be an antisense molecule including a modified antisense molecule, such as a morpholino antisense molecule. The polynucleotide can be modified, *e.g.*, it can be synthesized to have a morpholino backbone. If expressed, the polynucleotide preferably includes the necessary regulatory elements, such as a promoter, as known in the art. A specific example of a polypeptide is insulin.

The cargo moiety can be an organic molecule that is hydrophobic in aqueous media. The cargo moiety can also be a water-soluble monovalent or polyvalent cationic molecule, anionic, or net neutral at physiological pH.

The cargo moiety can be a drug, such as, a protein, a small peptide, a bioactive polynucleotide, an antibiotic, an antiviral, an anesthetic, an antipsychotic, an anti-infectious, an antifungal, an anticancer, an immunosuppressant, an immunostimulant, a steroidal anti-inflammatory, a non-steroidal anti-inflammatory, an antioxidant, an antidepressant which can be synthetically or naturally derived, a substance which supports or enhances mental function or inhibits mental deterioration, an anticonvulsant, an HIV protease inhibitor, a non-nucleophilic reverse transcriptase inhibitor, a cytokine, a tranquilizer, a mucolytic agent, a dilator, a vasoconstrictor, a decongestant, a leukotriene inhibitor, an anti-cholinergic, an anti-histamine, a cholesterol lipid metabolism modulating agent, or a vasodilatory agent. The drug can also be any over the counter (non-prescription) medication. Examples include Amphotericin B, acyclovir, adriamycin, carbamazepine, ivermectin, melphalen, nifedipine, indomethacin, curcumin, aspirin, ibuprofen, naproxen, acetaminophen, rofecoxib, diclofenac, ketoprofin, meloxicam, nabumetone, estrogens, testosterones, steroids, phenytoin, ergotamines, cannabinoids, rapamycin, propanadid, propofol,

alphadione, echinomycin, miconazole, miconazole nitrate, ketoconazole, itraconazole, fluconazole, griseofulvin, clotrimazole, econazole, terconazole, butoconazole, oxiconazole, sulconazole, saperconazole, voriconazole, ciclopirox olamine, haloprogin, tolnaftate, naftifine, terbinafine hydrochloride, morpholines, flucytosine, natamycin, butenafine, undecylenic acid, Whitefield's ointment, propionic acid, and caprylic acid, clioquinol, selenium sulfide, teniposide, hexamethylmelamine, taxol, taxotere, 18-hydroxydeoxycorticosterone, prednisolone, dexamethazone, cortisone, hydrocortisone, piroxicam, diazepam, verapamil, vancomycin, tobramycin, teicoplanin, bleomycin, peptidolglycan, ristocetin, sialoglycoproteins, orienticin, avaporcin, helevecardin, galacardin, actinoidin, gentamycin, netilmicin, amikacin, kanamycin A, kanamycin B, neomycin, paromomycin, neamine, streptomycin, dihydrostreptomycin, apramycin, ribostamycin, spectinomycin, caspofungin, echinocandin B, aculeacin A, micafungin, anidulafungin, cilofungin, pneumocandin, geldanamycin, nystatin, rifampin, tyrphostin, a glucan synthesis inhibitor, vitamin A acid, mesalamine, risedronate, nitrofurantoin, dantrolene, etidronate, nicotine, amitriptyline, clomipramine, citalopram, dothepin, doxepin, fluoxetine, imipramine, lofepramine, mirtazapine, nortriptyline, paroxetine, reboxitine, sertraline, trazodone, venlafaxine, dopamine, St. John's wort, phosphatidylserine, phosphatidic acid, amastatin, antipain, bestatin, benzamidine, chymostatin, 3,4-dichloroisocoumarin, elastatinal, leupeptin, pepstatin, 1,10-phenanthroline, phosphoramidon, ethosuximide, ethotoin, felbamate, fosphenytoin, lamotrigine, levitiracetam, mephenytoin, methsuximide, oxcarbazepine, phenobarbital, phensuximide, primidone, topirimate, trimethadione, zonisamide, saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir.

An antifungal drug can be a polyene macrolide, tetraene macrolide, pentaenic macrolide, fluorinated pyrimidine, imidazole, azole, triazole, halogenated phenolic ether, thiocarbamate, allylamine, sterol inhibitor, and an agent that interpolates fungal cell wall components.

The drug can be a polypeptide such as cyclosporin, Angiotensin I, II and III, enkephalins and their analogs, ACTH, anti-inflammatory peptides I, II, III, bradykinin, calcitonin, b-endorphin, dinorphin, leucokinin, leutinizing hormone

releasing hormone (LHRH), insulin, neurokinins, somatostatin, substance P, thyroid releasing hormone (TRH) and vasopressin.

The drug can be an antigen, but is not limited to a protein antigen. The antigen can also be a carbohydrate or DNA. Examples of antigenic proteins include membrane proteins, carbohydrates, envelope glycoproteins from viruses, animal cell proteins, plant cell proteins, bacterial proteins, and parasitic proteins.

The cargo moiety can be a nutrient, including, but not limited to lycopene, micronutrients such as phytochemicals or zoochemicals, vitamins, minerals, fatty acids, amino acids, fish oils, fish oil extracts, and saccharides, herbal products, essential oils, or flavor agents. Specific examples include Vitamins A, B, B1, B2, B3, B12, B6, B-complex, C, D, E, and K, vitamin precursors, caroteniods, and beta-carotene, resveratrol, biotin, choline, inositol, ginko, lutein, zeaxanthine, quercetin, silibinin, perillyl alcohol, genistein, sulfurophane, essential fatty acids, including eicosapentanoic acid (EPA), gamma-3, omega-3, gamma-6, and omega-6 fatty acids, herbs, spices, and iron. Minerals include, but are not limited to boron, chromium, colloidal minerals, colloidal silver, copper, manganese, potassium, selenium, vanadium, vanadyl sulfate, calcium, magnesium, barium, iron and zinc.

As used herein, "micronutrient" is a nutrient that the body must obtain from outside sources. Generally micronutrients are essential to the body in small amounts.

The cargo moiety can be a saccharide or sweetener, *e.g.,* saccharine, isomalt, maltodextrine, aspartame, glucose, maltose, dextrose, fructose and sucrose. Flavor agents include oils, essential oils, or extracts, including but not limited to oils and extracts of cinnamon, vanilla, almond, peppermint, spearmint, chamomile, geranium, ginger, grapefruit, hyssop, jasmine, lavender, lemon, lemongrass, marjoram, lime, nutmeg, orange, rosemary, sage, rose, thyme, anise, basil, and black pepper, tea or tea extracts, an herb, a citrus, a spice or a seed.

In yet another aspect, the present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder which can be treated with one or more cargo moiety.

"Treatment", or "treating" as used herein, is defined as the application or administration of a therapeutic agent (*e.g.*, antibiotics) to a subject or patient, or

application or administration of a therapeutic agent to an isolated tissue or cell line from a subject or patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder, or the predisposition toward disease. "Treated," as used herein, refers to the disease or disorder being cured, healed, alleviated, relieved, altered, remedied, ameliorated improved or affected. For example, certain methods of treatment of the instant invention provide for administration of anti-inflammatory compositions, such that inflammation is lessened or alleviated.

The terms "cure," "heal," "alleviate," "relieve," "alter," "remedy," "ameliorate," "improve" and "affect" are evaluated in terms of a suitable or appropriate control. A "suitable control" or "appropriate control" is any control or standard familiar to one of ordinary skill in the art useful for comparison purposes. In one embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined prior to administration of a cargo moiety composition, as described herein. For example, the number of colony forming units can be determined prior to administering an antifungal composition of the invention to a host. In another embodiment, a "suitable control" or "appropriate control" is a value, level, feature, characteristic, property, etc. determined in a subject, *e.g.*, a control or normal subject exhibiting, for example, normal traits. In yet another embodiment, a "suitable control" or "appropriate control" is a predefined value, level, feature, characteristic, property, etc.

The methods of the present invention include methods of administering a cargo moiety to a host, wherein the cargo moiety is associated with a composition of the invention. The compositions of the present invention may be administered orally, nasally, topically, intravenously, transdermally, buccally, sublingually, rectally, vaginally or parenterally.

The present invention provides a method for treating a subject that would benefit from administration of a composition of the present invention. Any therapeutic indication that would benefit from a cargo moiety, *e.g.*, a drug or nutrient, can be treated by the methods of the invention. Accordingly, the present invention provides methods of treating a subject at risk for or having a disease or

disorder which can be treated with, for example, a protein, a small peptide, a bioactive polynucleotide, an antibiotic, an antiviral, an anesthetic, antipsychotic, an anti-infectious, an antifungal, an anticancer, an immunosuppressant, an immunostimulant, a steroidal anti-inflammatory, a non-steroidal anti-inflammatory, an antioxidant, an antidepressant which can be synthetically or naturally derived, a substance which supports or enhances mental function or inhibits mental deterioration, an anticonvulsant, an HIV protease inhibitor, a non-nucleophilic reverse transcriptase inhibitor, a cytokine, a tranquilizer, a mucolytic agent, a dilator, a vasoconstrictor, a decongestant, a leukotriene inhibitor, an anti-cholinergic, an anti-histamine, a cholesterol lipid metablolism modulating agent or a vasodilatory agent. The method includes the step of administering to the subject a composition of the invention, such that the disease or disorder is treated.

The disease or disorder can be, *e.g.*, inflammation, pain, infection, fungal infection, bacterial infection, viral infection, parasitic disorders, an immune disorder, genetic disorders, degenerative disorders, cancer, proliferative disorders, obesity, depression, hair loss, impotence, hypertension, hypotension, dementia, senile dementia, or malnutrition, acute and chronic leukemia and lymphoma, sarcoma, adenoma, carcinomas, epithelial cancers, small cell lung cancer, non-small cell lung cancer, prostate cancer, breast cancer, pancreatic cancer, hepatocellular carcinoma, renal cell carcinoma, biliary cancer, colorectal cancer, ovarian cancer, uterine cancer, melanoma, cervical cancer, testicular cancer, esophageal cancer, gastric cancer, mesothelioma, glioma, glioblastoma, pituitary adenomas, schizophrenia, obsessive compulsive disorder (OCD), bipolar disorder, Alzheimer's disease, Parkinson's disease, cell proliferative disorders, blood coagulation disorders, Dysfibrinogenaemia and hemophilia (A and B), autoimmune disorders, *e.g.*, systemic lupus erythematosis, multiple sclerosis, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, Grave's disease, allogenic transplant rejection, ankylosing spondylitis, psoriasis, scleroderma, uveitis, eczema, dermatological disorders, hyperlipidemia, hyperglycemia, and/or hypercholesterolemia.

The compositions and methods of the instant invention may also be used to promote greater health or quality of life, for example limit cholesterol uptake or regulate lipid metabolism, weight gain, hunger, aging, or growth. Cosmetic

effects such as wrinkle reduction, hair growth, pigmentation, or dermatologic disorders may also be treated. The compositions and methods may also treat hereditary disease such as cystic fibrosis or muscular dystrophy.

The compositions and methods of the instant invention may be used to treat a variety of inflammations, including headache, arthritis, rheumatoid arthritis, osteoarthritis, atherosclerosis, acute gout, acute or chronic soft tissue damage associated with, *e.g.*, a sports injury, tennis elbow, bursitis, tendonitis, acute or chronic back pain, such as a herniated disc, carpal tunnel syndrome, glomerulonephritis, carditis, ulcerative colitis, asthma, sepsis, and plantar fasciitis. The compositions and methods of the invention may also be used to relieve pain resulting from surgery or other medical procedure. The compositions and methods of the instant invention may further be used to treat a variety of fungal infections, including candida, *e.g.*, yeast infection, tinea, *e.g.*, Athlete's foot, pityriasis, thrush, cryptococcal meningitis, histoplasmosis, and blastomycosis.

The compositions and methods of the instant invention may also be used to treat a variety of bacterial infections, including but not limited to moderate to severe lower respiratory tract infections, skin infections, biliary tract infections, bone infections, antibiotic prophylaxis, pseudomembraneous enterocolitis, central nervous system infections (*e.g.*, meningitis and ventriculitis), intra-abdominal infections (*e.g.*, peritonitis), pneumonia, septicemia, soft tissue infections, neutropaenic sepsis, joint infections, infective endocartidis, and urinary tract infections.

Exemplary bacteria that may be treated with the antibiotic preparation of the present invention include, but are not limited to, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae, Streptococcus Group D, Clostridium perfringens, Haemophilus influenzae, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae.*

The compositions and methods of the present invention may be employed for treating a variety of fungal infections, including, but not limited to, asthma, chronic rhinosinusitis, allergic fungal sinusitis, sinus mycetoma, non-invasive fungus induced mucositis, non-invasive fungus induced intestinal mucositis, chronic otitis media, chronic colitis, inflammatory bowel diseases, ulcerative colitis, Crohn's disease, candidemia, intraabdominal abscesses, peritonitis, pleural

space infections, esophageal candidiasis and invasive aspergillosis. Exemplary fungi that can be treated using antifungal compositions of the invention include, without limitation, Absidia, Acinteobacter, Aspergillus flavus, Aspergillus fumigatus, Aspergillus glaucus, Aspergillus nidulans, Aspergillus terreus, Aspergillus versicolor, Alternaria, Basidiobolus, Bipolaris, Candida albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida lypolytica, Candida neoformans, Candida parapsilosis, Candida tropicalis, Cladosporium, Conidiobolus, Cunninahamella, Curvularia, Dreschlera, Enterobacter, Exserohilum, Fusarium, Klebsiella, Malbranchia, Paecilonvces, Penicillium, Pseudallescheria, Rhizopus, Schizophylum, Sporothrix, Acremonium, Arachniotus citrinus, Aurobasidioum, Beauveria, Chaetomium, Chryosporium, Epicoccum, Exophilia jeanselmei, Geotrichum, Oidiodendron, Phoma, Pithomyces, Rhinocladiella, Rhodoturula, Sagrahamala, Scolebasidium, Scopulariopsis, Ustilago, Trichoderma, and Zygomycete.

The above methods may be employed in the absence of other treatment, or in combination with other treatments. Such treatments can be started prior to, concurrent with, or after the administration of the compositions of the instant invention.

Accordingly, the methods of the invention can further include the step of administering a second treatment, such as for example, a second treatment for the disease or disorder or to ameliorate side effects of other treatments. Such second treatment can include, *e.g.*, radiation, chemotherapy, transfusion, operations (*e.g.*, excision to remove tumors), and gene therapy. Additionally or alternatively, further treatment can include administration of drugs to further treat the disease or to treat a side effect of the disease or other treatments (*e.g.*, anti-nausea drugs).

With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype"). Thus, another aspect of the invention

provides methods for tailoring an individual's prophylactic or therapeutic treatment according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

The language "therapeutically effective amount" is that amount necessary or sufficient to produce the desired physiologic response. The effective amount may vary depending on such factors as the size and weight of the subject, or the particular compound. The effective amount may be determined through consideration of the toxicity and therapeutic efficacy of the compounds by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it may be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to unaffected cells and, thereby, reduce side effects.

Subjects at risk for a disease or condition which can be prophylactically treated with the agents mentioned herein can be identified by, *e.g.*, any or a combination of diagnostic or prognostic assays known to those skilled in the art. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the disease or disorder, such that the disease or disorder is prevented or, alternatively, delayed in its progression.

Another aspect of the invention pertains to methods of administering compositions of the invention for therapeutic purposes. In one embodiment, the present invention provides a method for treating a subject that would benefit from administration of a composition of the present invention. Any therapeutic indication that would benefit from administration of a composition of the invention can be treated by the methods of the invention. The present invention provides methods of treating a subject at risk for or having a disease or disorder that can be treated with one or more cargo moieties. The method includes the step

of administering to the subject a composition of the invention, such that the disease or disorder is prevented, ameliorated, terminated or delayed in its progression. The disease or disorder can be any of the diseases or disorders discussed herein.

The invention also pertains to uses of the compositions of the invention for prophylactic and therapeutic treatments as described infra. Accordingly, the compounds of the present invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the compositions of the invention and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants may also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants, which may also be present in formulations of therapeutic compounds of the invention, include water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Furthermore, the present invention can further include one or more additional agents, including water, antimicrobial agents, plasticizing agents, flavoring agents, surfactants, stabilizing agents, emulsifying agents, thickening agents, binding agents, coloring agents, sweeteners, fragrances, and the like.

Suitable antimicrobial agents include triclosan, cetyl pyridium chloride, domiphen bromide, quaternary ammonium salts, zinc compounds, sanguinarine,

fluorides, alexidine, octonidine, EDTA, and essential oils such as thymol, methyl salicylate, menthol and eucalyptol.

Suitable plasticizing agents include, for example, polyols such as sugars, sugar alcohols, or polyethylene glycols (PEGs), urea, glycol, propylene glycol, triethyl citrate, dibutyl or dimethyl phthalate, monoacetin, diacetin or triacetin.

Suitable surfactants include pluronic acid, sodium lauryl sulfate, mono and diglycerides of fatty acids and polyoxyethylene sorbitol esters, such as, Atmos 300 and Polysorbate 80. Suitable stabilizing agents include xanthan gum, locust bean gum, guar gum, and carrageenan. Suitable emulsifying agents include triethanolamine stearate, quaternary ammonium compounds, acacia, gelatin, lecithin, bentonite, veegum, and the like. Suitable thickening agents include methylcellulose, carboxyl methylcellulose, and the like. Suitable binding agents include starch.

Suitable sweeteners that can be included are those well known in the art, including both natural and artificial sweeteners. Suitable sweeteners include water-soluble sweetening agents such as monosaccharides, disaccharides and polysaccharides; water-soluble artificial sweeteners such as soluble saccharin salts, cyclamate salts, or the free acid form of saccharin, and the like; dipeptide based sweeteners, such as L-aspartic acid derived sweeteners; water-soluble sweeteners derived from naturally occurring water-soluble sweeteners, such as a chlorinated derivative of ordinary sugar (sucrose), known, under the product description of sucralose; and protein based sweeteners such as thaumatoccous danielli (Thaumatin I and II).

In general, an effective amount of auxiliary sweetener is utilized to provide the level of sweetness desired for a particular composition, and this amount will vary with the sweetener selected. This amount will normally be 0.01% to about 10% by weight of the composition when using an easily extractable sweetener.

The flavorings that can be used include those known to the skilled artisan, such as natural and artificial flavors. These flavorings may be chosen from synthetic flavor oils and flavoring aromatics, and/or oils, oleo resins and extracts derived from plants, leaves, flowers, fruits and so forth, and combinations thereof. Representative flavor oils include: spearmint oil, cinnamon oil, peppermint oil, clove oil, bay oil, thyme oil, cedar leaf oil, oil of nutmeg, oil of sage, and oil of

bitter almonds. Also useful are artificial, natural or synthetic fruit flavors such as vanilla, chocolate, coffee, cocoa and citrus oil, and fruit essences. These flavorings can be used individually or in admixture. Flavorings such as aldehydes and esters including cinnamyl acetate, cinnamaldehyde, citral, diethylacetal, dihydrocarvyl acetate, eugenyl formate, p-methylanisole, and so forth may also be used. Generally, any flavoring or food additive, such as those described in Chemicals Used in Food Processing, publication 1274 by the National Academy of Sciences, pages 63-258, may be used.

The amount of flavoring employed is normally a matter of preference subject to such factors as flavor type, individual flavor, and strength desired. Thus, the amount may be varied in order to obtain the result desired in the final product. Such variations are within the capabilities of those skilled in the art without the need for undue experimentation.

The compositions of this invention can also contain coloring agents or colorants. The coloring agents are used in amounts effective to produce the desired color. The coloring agents useful in the present invention include pigments such as titanium dioxide, which may be incorporated in amounts of up to about 5 wt %, and preferably less than about 1 wt %. Colorants can also include natural food colors and dyes suitable for food, drug and cosmetic applications. These colorants are known as FD&C dyes and lakes. A full recitation of all FD&C and D&C dyes and their corresponding chemical structures may be found in the Kirk-Othmer Encyclopedia of Chemical Technology, Volume 5, Pages 857-884, which text is accordingly incorporated herein by reference.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which may be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of 100%, this amount will range from about 1% to about 99% of active ingredient, preferably from about 5% to about 70%, most preferably from about 10% to about 30%.

Methods of preparing these formulations or compositions include the step of bringing into association a composition of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a composition of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, gelcaps, crystalline substances, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, gel, partial liquid, spray, nebulae, mist, atomized vapor, aerosol, tincture, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) or as mouth washes and the like, each containing a predetermined amount of a composition of the present invention as an active ingredient. A composition of the present invention may also be administered as a bolus, electuary, or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents.

In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such

excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered composition moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes or microspheres.

They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which may be dissolved in sterile water, or some other sterile injectable medium immediately before use.

These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which may be used include polymeric substances and waxes. The active ingredient may also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert dilutents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as

ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert dilutents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented in liquid or aerosol form, or as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound. Liquid or aerosol forms include, but are not limited to, gels, pastes, ointments, salves, creams, solutions, suspensions, partial liquids, sprays, nebulaes, mists, atomized vapors, and tinctures. Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Formulations of the pharmaceutical compositions of the invention for nasal administration can be in solid, liquid, or aerosol form (*e.g.*, powder, crystalline substance, gel, paste, ointment, salve, cream, solution, suspension, partial liquid, spray, nebulae, irrigant, wash, mist, atomized vapor or tincture).

Dosage forms for the topical or transdermal administration of a composition of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The composition may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an composition of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays may contain, in addition to a composition of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays may additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a composition of the present invention to the body. Such dosage forms may be made by dissolving or dispersing the composition in the proper medium. Absorption enhancers may also be used to increase the flux of the composition across the skin. The rate of such flux may be controlled by either providing a rate controlling membrane or dispersing the composition in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like are also within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise a composition of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a cargo moiety, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable

to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating a composition of the invention in the desired amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the composition into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the compositions of the invention plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release may be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the composition can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the composition in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium

stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compositions of the invention also can be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the compositions of the invention are prepared with carriers that will protect the composition against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity

of a composition calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the composition and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such a composition for the treatment of individuals.

The compositions can be included in a container, pack, or dispenser together with instructions for administration.

The compositions can be included in a container along with one or more additional compounds or compositions and instructions for use. For example, the invention also provides for packaged pharmaceutical products containing two agents, each of which exerts a therapeutic effect when administered to a subject in need thereof. A pharmaceutical composition may also comprise a third agent, or even more agents yet, wherein the third (and fourth, etc.) agent can be another agent against the disorder, such as a cancer treatment (*e.g.*, an anticancer drug and/or chemotherapy) or an HIV cocktail. In some cases, the individual agents may be packaged in separate containers for sale or delivery to the consumer. The agents of the invention may be supplied in a solution with an appropriate solvent or in a solvent-free form (*e.g.*, lyophilized). Additional components may include acids, bases, buffering agents, inorganic salts, solvents, antioxidants, preservatives, or metal chelators. The additional kit components are present as pure compositions, or as aqueous or organic solutions that incorporate one or more additional kit components. Any or all of the kit components optionally further comprise buffers.

The compositions of the instant invention may be used for delivering cargo moieties to food and drinks to be consumed by humans or other animals. For example, animal food (*e.g.*, human, cat, dog, fish, and bird food), can include the compositions of the present invention to stably deliver vitamins, minerals or other nutrients, as well as medications, *e.g.*, allergy medications and/or additional cargo moieties. The compositions of the present invention may be added to pet or domestic animal feed, such as fish food and food for fowl, cattle, and horses. The vehicles can be added at any step of the food preparation. For example, the formulations of the invention can be added at any point in the methods described

in WO 02/44026, incorporated herein by this reference. Similarly, the compositions and methods of the invention may be employed in food or drink to be consumed by humans, *e.g.*, in nutrient bars or drinks, cereals, breads, and snack foods. Accordingly, the preparations of the invention allow for the production of stable, convenient preparations of micronutrients in processed foods, such as fast foods. Typically, potentially beneficial micronutrients, *e.g.*, omega fatty acids and antioxidants, can be destroyed during food manufacture and storage. The formulations of the invention may protect micronutrients and other cargo moieties, thus increasing the nutritional and/or medicinal value of the food.

The compositions and methods of the present invention can be added to foods that are baked or cooked, such as cakes, muffins, pasta noodles, soups, cereals, chips, candy and cookies. In some embodiments, the compositions are used in candy, such as candy bars, *e.g.*, chocolate bars. For example, omega fatty acid-compositions can be incorporated into a chocolate bar.

The present invention is useful in a variety of foods, including, dried food and beverage mixes, ready-to-drink and eat beverages and foods. These include baked good mixes and baked goods (*e.g.*, bread, cakes, brownies, muffins, cookies, pastries, pies, crackers, pie crusts), fried snacks derived from potatoes, corn, wheat and other grains (*e.g.*, potato chips, corn chips, tortilla chips), other fried farinaceous snack foods (*e.g.*, french fries, doughnuts, fried chicken), dairy products and artificial dairy products (*e.g.*, butter, ice cream and other fat-containing frozen desserts, yogurt, and cheeses, including natural cheeses, processed cheeses, cream cheese, cottage cheese, cheese foods and cheese spread, milk, cream, sour cream, butter milk, and coffee creamer), cereal products, baby foods or formulas, puddings, ice cream, dips, syrups, pie and other dessert fillings, frostings, emulsified spreads such as salad dressings, mayonnaise and margarines, various kinds of soups, dips, sauces and gravies. The preparations can include additional agents typically found in food preparations, such as coloring agents, flavoring agents, edible acids, preservatives, and the like.

The compositions of the present invention may be added to the food products in a crystallized or emulsion form at any stage of the manufacturing process. The compositions may be added at a stage and in a manner where the integrity of the delivery vehicle is maintained until ingestion, or final preparation

of the food product by the consumer. Another alternative, however, can be to use the compositions to maintain the stability of the agent until incorporation into the product, so activity can be maintained during storage and shipping. For example, food and drink mixes can contain compositions of the present invention that de-precipitate in whole or in part when reconstituted prior to ingestion. In this case, the compositions maintain the stability and integrity of the cargo moiety until ingestion so that the ingested food or drink contains the cargo moiety in a non-degraded state.

Yet another alternative is to deliver the formulations themselves to consumers or professionals, for direct addition to food products, *e.g.*, medicament, nutrient crystals, additives, supplements, or emulsions, such that one can vary the concentration as desired.

The compositions of the present invention may also be added to a carrier for use as a topical treatment on the skin. Suitable carriers would remain on the skin for an extended period of time, and be resistant to perspiration or immersion in water. Thus, for example, the formulations may be added to topical applications of medicaments, moisturizers, deodorants, balms, fragrances, sunscreens, and the like.

Additional examples of formulations that may include the compositions of the invention include, but are not limited to, hair care products, skin care products, personal care products, personal cleansing products, lotions, fragrances, sprays, perfumes, cosmetics, toothpastes, tooth whiteners, cleaners, bar soap, liquid soap, body wash, baby wash, makeup, hair color, shampoos, conditioners, styling products, balms, creams, solutions, gels and solids. Thus, for example, shampoos, conditioners and the like may contain the compositions of the invention loaded with vitamins, moisturizers, perfumes, medications, etc.

The compositions of the invention may also be added to cleansers which do not typically have prolonged direct contact with the skin. These formulations would be advantageous for, *i.e.*, the incorporation of perfumes, moisturizers or other such cargo moieties into fabric or for the introduction of an antibacterial agent to dishes. Examples include, but are not limited to, laundry detergent, pre-treating formulations, dryer sheets, fabric softener, and dishwashing detergent.

Compositions of the present invention may also be added to paper products for the topical application of cargo moieties to skin. Examples of paper products that can include compositions of the invention include baby care products, *i.e*, diapers or baby wipes, tissues, toilet paper, antibacterial or antiperspirant towelettes, napkins, paper towels, bandaids, gauze pads, and feminine hygiene products.

Exemplification

Example 1: Formation of Rigid Liposomes

Rigid liposomes were formed using distearoyl phosphatidylserine (DSPS) from Avanti Polar Lipids (Birmingham, AL), having a transition temperature of 68°C. Rhodamine-labeled Dextran (Rho-Dextran) in water was added to the DSPS in water at 0.1% by weight at room temperature.

The sample was heated to 70°C over a time period of 15 minutes while periodically vortexing to thoroughly mix the sample. Microscopic observations were made as the sample was warmed (Figures 1A and 1B). As the heating process began, the DSPS crystals slowly began to disappear and the tube became clear. After heating the sample above its transition temperature for approximately 15 minutes, the phospholipids were observed to form liposomes containing Rho-Dextran (Figure 1B).

It was determined using fluorescence microscopy that the Rho-Dextran was within the DSPS liposomes, not free in the external aqueous space. Once the liposomes were formed, and the Rho-Dextran was found in the internal aqueous space of the DSPS liposomes, the sample was then cooled to 37°C, at which point the liposomes became rigid. Calcium was then added causing an interaction that caused the composition to resemble a bunch or cluster of grapes (Figure 1C). It appeared as though the calcium formed a cationic bridge between the rigid liposomes, pulling them in together and forming a cluster of liposomes.

In the formation of cochleates from diolyl phosphatidylserine (DOPS), which does not have a high transition temperature, the addition of EDTA chelates the calcium, such that the cochleate structure is unrolled and/or unstacked. In contrast, when EDTA was added to the DSPS sample at 37°C, no changes were

observed (Figure 1D). The sample remained rigid, and did not allow the EDTA to interact with the calcium.

When heated above the transition temperature of 68°C, however, the cationic matrix was observed to open up and the DSPS liposomes were observed become fluid.

Example 2: Formation of Encochleated Rigid Liposomes

Rigid DSPS liposomes were formed in accordance with Example 1, except that the matrix was formed by forming a precipitate of DOPS and calcium. The DSPS lipid (10mg/ml) containing Rhodamine-labeled dextran, was added to water and heated above 68°C while periodically vortexing. The heating of the lipid above its transition temperature allowed for the phospholipids to become fluid and small liposomes formed. The DSPS liposomes were then cooled to room temperature, at which point they became rigid as in Example 1. DSPS liposomes were added to pre-formed DOPS liposomes in water at a ratio of 1 to 10 by weight, respectively. The sample was vortexed to ensure the rigid DSPS liposomes were evenly distributed, at which point the sample was observed microscopically. Microscopy of the sample revealed a population of two types of liposomes, those that were fluid and those that were rigid. The rigid DSPS liposomes were somewhat smaller than those composed of DOPS. After microscopic observations indicated a heterogeneous population, calcium (0.1M) was added by dropwise addition while vortexing to allow formation of the cochleates. Upon addition of calcium, the DOPS liposomes, which were surrounding the DSPS rigid liposomes, interacted with the calcium. The calcium interaction caused a restructuring of the DOPS liposomes forcing out the water of hydration, but did not appear to affect the structure of the rigid DSPS liposomes. Microscopic observation indicated that DOPS cochleates were formed, and appeared to be structurally similar to those that were prepared with the standard DOPS. When the sample was observed by fluorescence, it appeared that the rigid DSPS clusters liposomes were disposed in a DOPS cochleate matrix.

Figure 2A is an image of the formulation observed under phase contrast optical microscopy. Using both fluorescence and phase contrast microscopy, the rigid DSPS Rho-Dextran liposomes can be observed within the DOPS cochleate

structure as shown in Figure 2B. Turning down the phase contrast light, numerous fluorescent DSPS Rho-Dextran liposomes were observed within the cochleate structure as captured in Figure 2C.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim:

1. A composition comprising a plurality of liposomes having a high transition temperature at least partially disposed in a matrix.

2. The composition of claim 1, wherein the transition temperature of the plurality of liposomes is at least about 30°C.

3. The composition of claim 1, wherein the transition temperature of the plurality of liposomes is at least about 45°C.

4. The composition of claim 1, wherein the transition temperature of the plurality of liposomes is at least about 60°C.

5. The composition of claim 1, wherein plurality of liposomes comprises at least one phospholipid selected from the group consisting of: distearoyl phosphatidylserine (DSPS), distearoyl phosphatidylcholine (DSPC), dipalmitoyl phosphatidic acid (DPPA), dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylethanolamine (DPPE), dipalmitoyl phosphatidylserine (DPPS), dipalmitoyl phosphatidylgycerol (DPPG) dimyristoyl phosphatidylethanolamine (DMPE), dioleoyl phosphatidic acid (DOPA), and dimyristoyl phosphatidylserine (DMPS).

6. The composition of claim 1, wherein the plurality of liposomes comprises a negatively charged lipid, and the matrix is a cationic bridge between a multivalent cation and at least a portion of the negatively charged lipid.

7. The composition of claim 1, wherein the matrix is a precipitate comprising a multivalent cation and a negatively charged lipid.

8. The composition of claim 1, wherein the matrix comprises a cochleate comprising a multivalent cation and a negatively charged lipid.

9. The composition of any of claims 1-8, wherein the composition comprises a cargo moiety.

10. The composition of claim 9, wherein the cargo moiety is associated with at least a portion of the plurality liposomes.

11. The composition of claim 10, wherein the cargo moiety is disposed within at least a portion of the plurality liposomes.

12. The composition of claim 9, wherein the cargo moiety is associated with the matrix.

13. The composition of claim 9, wherein the composition comprises at least one additional cargo moiety.

14. The composition of claim 9, wherein the cargo moiety is at least one member selected from the group consisting of a vitamin, a mineral, a nutrient, a micronutrient, an amino acid, a toxin, a microbicide, a microbistat, a co-factor, an enzyme, a polypeptide, a polypeptide aggregate, a polynucleotide, a lipid, a carbohydrate, a nucleotide, a starch, a pigment, a fatty acid, a saturated fatty acid, a monounsaturated fatty acid, a polyunsaturated fatty acid, a flavoring, an essential oil or extract, a hormone, a cytokine, a virus, an organelle, a steroid or other multi-ring structure, a saccharide, a metal, a metabolic poison, an antigen, an imaging agent, a porphyrin, a tetrapyrrolic pigment, and a drug.

15. The composition of claim 14, wherein the drug is at least one member selected from the group consisting of a protein, a small peptide, a bioactive polynucleotide, an antibiotic, an antiviral, an anesthetic, an antipsychotic, an anti-infectious, an antifungal, an anticancer, an immunosuppressant, an immunostimulant, a steroidal anti-inflammatory, a non-steroidal anti-inflammatory, an antioxidant, an antidepressant which can be synthetically or naturally derived, a substance which supports or enhances mental

function or inhibits mental deterioration, an anticonvulsant, an HIV protease inhibitor, a non-nucleophilic reverse transcriptase inhibitor, a cytokine, a tranquilizer, a mucolytic agent, a dilator, a vasoconstrictor, a decongestant, a leukotriene inhibitor, an anti-cholinergic, an anti-histamine, a cholesterol lipid metabolism modulating agent, and a vasodilatory agent.

16. The composition of claim 14, wherein the drug is at least one member selected from the group consisting of Amphotericin B, acyclovir, adriamycin, carbamazepine, ivermectin, melphalen, nifedipine, indomethacin, curcumin, aspirin, ibuprofen, naproxen, acetaminophen, rofecoxib, diclofenac, ketoprofin, meloxicam, nabumetone, estrogens, testosterones, steroids, phenytoin, ergotamines, cannabinoids, rapamycin, propanadid, propofol, alphadione, echinomycin, miconazole, miconazole nitrate, ketoconazole, itraconazole, fluconazole, griseofulvin, clotrimazole, econazole, terconazole, butoconazole, oxiconazole, sulconazole, saperconazole, voriconazole, ciclopirox olamine, haloprogin, tolnaftate, naftifine, terbinafine hydrochloride, morpholines, flucytosine, natamycin, butenafine, undecylenic acid, Whitefield's ointment, propionic acid, and caprylic acid, clioquinol, selenium sulfide, teniposide, hexamethylmelamine, taxol, taxotere, 18-hydroxydeoxycorticosterone, prednisolone, dexamethazone, cortisone, hydrocortisone, piroxicam, diazepam, verapamil, vancomycin, tobramycin, teicoplanin, bleomycin, peptidolglycan, ristocetin, sialoglycoproteins, orienticin, avaporcin, helevecardin, galacardin, actinoidin, gentamycin, netilmicin, amikacin, kanamycin A, kanamycin B, neomycin, paromomycin, neamine, streptomycin, dihydrostreptomycin, apramycin, ribostamycin, spectinomycin, caspofungin, echinocandin B, aculeacin A, micafungin, anidulafungin, cilofungin, pneumocandin, geldanamycin, nystatin, rifampin, tyrphostin, a glucan synthesis inhibitor, vitamin A acid, mesalamine, risedronate, nitrofurantoin, dantrolene, etidronate, nicotine, amitriptyline, clomipramine, citalopram, dothepin, doxepin, fluoxetine, imipramine, lofepramine, mirtazapine, nortriptyline, paroxetine, reboxitine,

sertraline, trazodone, venlafaxine, dopamine, St. John's wort, phosphatidylserine, phosphatidic acid, amastatin, antipain, bestatin, benzamidine, chymostatin, 3,4-dichloroisocoumarin, elastatinal, leupeptin, pepstatin, 1,10-phenanthroline, phosphoramidon, ethosuximide, ethotoin, felbamate, fosphenytoin, lamotrigine, levitiracetam, mephenytoin, methsuximide, oxcarbazepine, phenobarbital, phensuximide, primidone, topirimate, trimethadione, zonisamide, saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir.

17. The composition of claim 14, wherein the polynucleotide is at least one member selected from the group consisting of a deoxyribonucleic acid (DNA) molecule, a ribonucleic acid (RNA) molecule, small interfering RNA (siRNA), a ribozyme, an antisense molecule, a morpholino and a plasmid.

18. The composition of claim 14, wherein the DNA is transcribed to yield a ribonucleic acid.

19. The composition of claim 18, wherein the ribonucleic acid is translated to yield a biologically active polypeptide.

20. The composition of claim 14, wherein the polypeptide is at least one member selected from the group consisting of cyclosporin, Angiotensin I, II, or III, enkephalins and their analogs, ACTH, anti-inflammatory peptides I, II, or III, bradykinin, calcitonin, beta-endorphin, dinorphin, leucokinin, leutinizing hormone releasing hormone (LHRH), insulin, neurokinins, somatostatin, substance P, thyroid releasing hormone (TRH), and vasopressin.

21. The composition of claim 14, wherein the antigen is at least one member selected from the group consisting of a membrane protein, a carbohydrate, envelope glycoproteins from viruses, an animal cell protein, a plant cell protein, a bacterial protein and a parasitic protein.

22. The method of claim 14, wherein the nutrient is at least one member selected from the group consisting of vitamins, minerals, fatty acids, amino acids, fish oils, fish oil extracts, resveratrol, biotin, choline, inositol, ginko, saccharides, a phytochemical or zoochemical, beta-carotene, lutein, zeaxanthine, quercetin, silibinin, perillyl alcohol, genistein, sulfurophane, lycopene, essential fatty acids, eicosapentanoic acid (EPA), gamma-3, omega-3, gamma-6, and omega-6 fatty acids.

23. The composition of claim 14, wherein the vitamin is at least one member selected from the group consisting of vitamins A, B, B1, B2, B3, B12, B6, B-complex, C, D, E, and K, vitamin precursors, caroteniods, and beta-carotene.

24. The composition of claim 14, wherein the mineral is at least one member selected from the group consisting of boron, chromium, colloidal minerals, colloidal silver, copper, manganese, potassium, selenium, vanadium, vanadyl sulfate, calcium, magnesium, barium, iron and zinc.

25. The composition of claim 14, wherein the fatty acid is at least one member selected from the group consisting of monounsaturated, polyunsaturated and saturated fatty acids.

26. The composition of claim 14, wherein the saccharide or sweetener is at least one member selected from the group consisting of saccharine, isomalt, maltodextrine, aspartame, glucose, maltose, dextrose, fructose and sucrose.

27. The composition of claim 14, wherein the flavor substance is an essential oil or an extract.

28. The composition of claim 14, wherein the flavor substance is selected from the group consisting of oils and extracts of cinnamon, vanilla,

almond, peppermint, spearmint, chamomile, geranium, ginger, grapefruit, hyssop, jasmine, lavender, lemon, lemongrass, marjoram, lime, nutmeg, orange, rosemary, sage, rose, thyme, anise, basil, black pepper and tea or tea extracts.

29. The composition of claim 28, wherein the extract is from at least one member selected from the group consisting of an herb, a citrus, a spice and a seed.

30. A pharmaceutical composition comprising the composition of any of claims 1-29 and a pharmaceutically acceptable carrier.

31. A method of treating a subject that can benefit from administration of a cargo moiety comprising administering the composition of any one of claims 9-29 such that the cargo moiety is administered to the subject.

32. The method of treatment according to claim 31, wherein the administration is by a mucosal or a systemic route.

33. The method of treatment according to claim 31, wherein the administration is a mucosal route selected from the group consisting of oral, intranasal, intraocular, intrarectal, intravaginal, and intrapulmonary.

34. The method of treatment according to claim 31, wherein the administration is by a systemic route selected from the group consisting of intravenous, intramuscular, subcutaneous, transdermal, and intradermal.

35. The method of claim 31, wherein the cargo moiety is administered to treat inflammation, pain, infection, fungal infection, bacterial infection, viral infection, parasitic disorders, an immune disorder, genetic disorders, degenerative disorders, cancer, proliferative disorders, obesity, depression, hair loss, impotence, hypertension, hypotension, dementia, senile dementia, or malnutrition, acute and chronic leukemia and lymphoma,

sarcoma, adenoma, carcinomas, epithelial cancers, small cell lung cancer, non-small cell lung cancer, prostate cancer, breast cancer, pancreatic cancer, hepatocellular carcinoma, renal cell carcinoma, biliary cancer, colorectal cancer, ovarian cancer, uterine cancer, melanoma, cervical cancer, testicular cancer, esophageal cancer, gastric cancer, mesothelioma, glioma, glioblastoma, pituitary adenomas, schizophrenia, obsessive compulsive disorder (OCD), bipolar disorder, Alzheimer's disease, Parkinson's disease, cell proliferative disorders, blood coagulation disorders, Dysfibrinogenaemia and hemophilia (A and B), autoimmune disorders, *e.g.*, systemic lupus erythematosis, multiple sclerosis, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, Grave's disease, allogenic transplant rejection, ankylosing spondylitis, psoriasis, scleroderma, uveitis, eczema, dermatological disorders, hyperlipidemia, hyperglycemia, or hypercholesterolemia.

36. A method of forming a composition comprising the step of forming a matrix at least partially about a plurality of liposomes having a high transition temperature.

37. The method of claim 36, wherein the plurality of liposomes comprise an anionic lipid and the matrix comprises a multivalent cation.

38. The method of claim 36, wherein the matrix comprises a multivalent cation forming a cationic bridge between a portion of the plurality of liposomes.

39. The method of claim 36, wherein the matrix comprises a precipitate of a multivalent cation and a second plurality of liposomes.

40. The method of claim 36, wherein the matrix comprises a cochleate comprising a multivalent cation and a second plurality of liposomes.

Figure 1

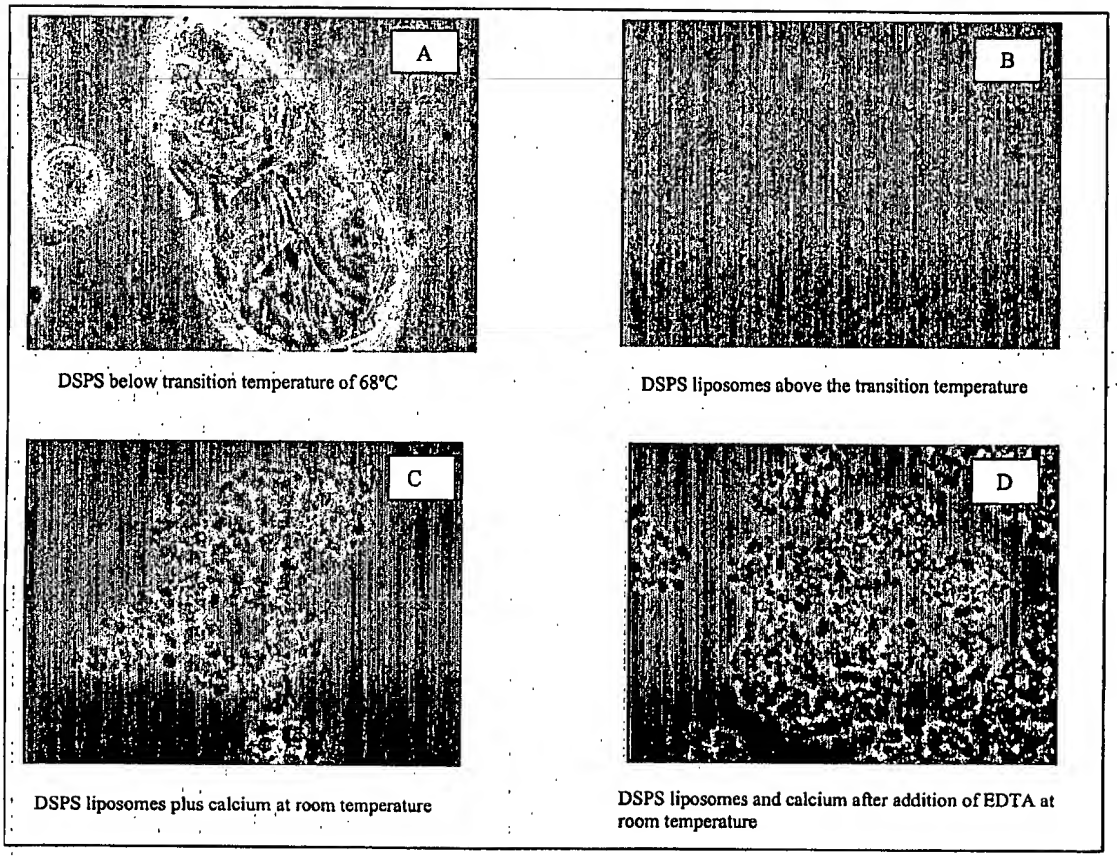
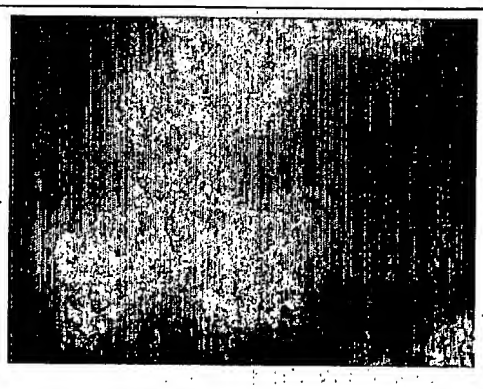
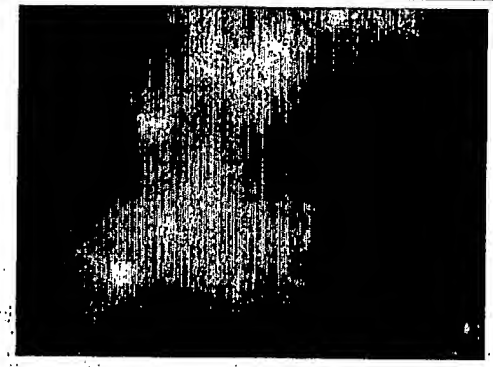

A
DSPS below transition temperature of 68°C
B
DSPS liposomes above the transition temperature
C
DSPS liposomes plus calcium at room temperature
D
DSPS liposomes and calcium after addition of EDTA at room temperature

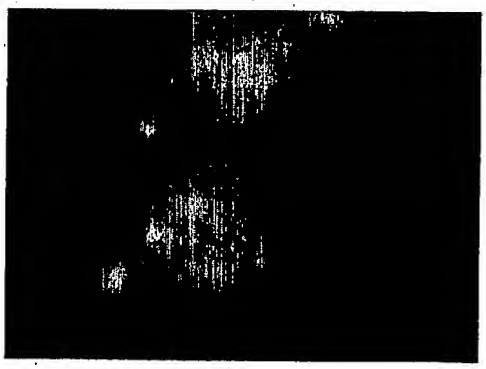
Figure 2



A. Phase Contrast of DOPS cochleates containing DSPS Rho-Dextran liposomes



B. Phase contrast and fluroescence microscopy of DOPS cochleates containing DSPS Rho-Dextran liposomes



C. Fluorescence microscopy of DOPS cochleates containing DSPS Rho-Dextran liposomes

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/042927

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/127 A61K47/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAMANI KARTHIK ET AL: "Fluorescence properties of Laurdan in cochleate phases." BIOCHIMICA ET BIOPHYSICA ACTA. 3 DEC 2003, vol. 1618, no. 1, 3 December 2003 (2003-12-03), pages 67-78, XP002325714 ISSN: 0006-3002 the whole document abstract page 69, column 2, paragraph 2 -/--	1-40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
22 April 2005	10/05/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Luangkhot, N

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUI S W ET AL: "IDENTIFICATION OF PHOSPHATIDYL SERINE AND PHOSPHATIDYL CHOLINE IN CALCIUM INDUCED PHASE SEPARATED DOMAINS" BIOCHEMISTRY, vol. 22, no. 14, 1983, pages 3511-3516, XP001206143 ISSN: 0006-2960 the whole document abstract page 3519, column 1, line 20 - column 2, line 1	1-40
X	KOUAOUCI R ET AL: "Calcium-induced lateral phase separations in phosphatidylcholine-phosphatidic acid mixtures. A Raman spectroscopic study." BIOCHEMISTRY. 3 DEC 1985, vol. 24, no. 25, 3 December 1985 (1985-12-03), pages 7132-7140, XP002325716 ISSN: 0006-2960 the whole document abstract figures	1-40
X	GRAHAM I ET AL: "Kinetics and thermodynamics of calcium-induced lateral phase separations in phosphatidic acid containing bilayers." BIOCHEMISTRY. 3 DEC 1985, vol. 24, no. 25, 3 December 1985 (1985-12-03), pages 7123-7131, XP001206146 ISSN: 0006-2960 the whole document abstract figures	1-40
X	FLACH C R ET AL: "A new infrared spectroscopoic marker for cochleate phases in phosphatidylserine-containing model membranes." BIOPHYSICAL JOURNAL. APR 1993, vol. 64, no. 4, April 1993 (1993-04), pages 1113-1121, XP009046737 ISSN: 0006-3495 the whole document abstract	1-40

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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